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NEWS EXPRESS JUNE 30 CURRENT WINDOWS VERSION IS V8.01b, CURRENT
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FILE 'SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006

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=> e lukyanov, s/au

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| E2 | 2 | LUKYANOV ZV/AU |
| E3 | 0 --> | LUKYANOV, S/AU |
| E4 | 1 | LUKYANOVA A A/AU |
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| E6 | 1 | LUKYANOVA A I/AU |
| E7 | 2 | LUKYANOVA A M/AU |
| E8 | 7 | LUKYANOVA A P/AU |
| E9 | 2 | LUKYANOVA A S/AU |
| E10 | 6 | LUKYANOVA A V/AU |
| E11 | 1 | LUKYANOVA C N/AU |
| E12 | 3 | LUKYANOVA E/AU |

=> s cnidarian and (anthozoan)

L1 123 CNIDARIAN AND (ANTHOZOAN)

=> s l2 and (nucleic acid0

UNMATCHED LEFT PARENTHESIS 'AND (NUCLEIC'

The number of right parentheses in a query must be equal to the number of left parentheses.

=> s l2 and nucleic acid

L2 NOT FOUND

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=> s 11 and nucleic acid

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SEARCH ENDED BY USER
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(FILE 'HOME' ENTERED AT 11:08:06 ON 22 SEP 2006)

FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCPLUS, WPIDS, FSTA, JICST-EPLUS,
SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006
E LUKYANOV, S/AU

L1 123 S CNIDARIAN AND (ANTHOZOAN)

=> s l1 and (non-bioluminescent)
L2 11 L1 AND (NON-BIOLUMINESCENT)

=> s l1 and (non-pennatulacean)
L3 5 L1 AND (NON-PENNATULACEAN)

=> d l3 ti abs ibib tot

L3 ANSWER 1 OF 5 HCPLUS COPYRIGHT 2006 ACS on STN
TI Rapidly maturing fluorescent proteins and methods for using the same
AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as
well as non-aggregating versions thereof (and mutants thereof) as well as
the proteins encoding the same, are provided. The proteins of interest
are proteins that are fluorescent, where this feature arises from the
interaction of two or more residues of the protein. The subject proteins
are further characterized in that, in certain embodiments, they are
mutants of wild type proteins that are obtained either from
non-bioluminescent Cnidarian, e.g., Anthozoan, species
or are obtained from Anthozoan non-
Pennatulacean (sea pen) species. In certain embodiments, the
subject proteins are mutants of wild type Discosoma sp. red fluorescent
protein. Also of interest are proteins that are substantially similar to,
or mutants of, the above specific proteins. Also provided are fragments
of the nucleic acids and the peptides encoded thereby, as well as
antibodies to the subject proteins and transgenic cells and organisms.
The subject protein and nucleic acid compns. find use in a variety of
different applications. Finally, kits for use in such applications, e.g.,
that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:588435 HCPLUS
DOCUMENT NUMBER: 143:112117
TITLE: Rapidly maturing fluorescent proteins and methods for
using the same
INVENTOR(S): Bevis, Brooke; Glick, Benjamin
PATENT ASSIGNEE(S): The University of Chicago, USA
SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of Appl.
No. PCT/US02/40539.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| US 2005149994 | A1 | 20050707 | US 2004-844064 | 20040511 |
| WO 2003054158 | A2 | 20030703 | WO 2002-US40539 | 20021218 |
| WO 2003054158 | A3 | 20031204 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
 PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,
 UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
 KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
 FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ,
 CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-341723P P 20011219
 WO 2002-US40539 A2 20021218

L3 ANSWER 2 OF 5 HCPLUS COPYRIGHT 2006 ACS on STN
 TI Novel Cnidarian and Anthozoan chromo/fluoroproteins
 and cDNA encoding them

AB DNA encoding novel chromo/fluoroproteins as well as the encoded proteins are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. Specific proteins of interest include the following specific proteins: Heteractis crispa hcriGFP; Dendronephthya dendGFP; Zoanthus zoanRFP; Scolymia cubensis scubGFP1 and scubGFP2; Ricordea florida rfloRFP, rfloGFP2, and rfloGFP; Montastraea cavernosa mcavRFP, mcavGFP, and mcavGFP2; Condylactis gigantea cgigGFP; Agaricia fragilis afraGFP; and Montastraea annularis mannFP. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:122690 HCPLUS
 DOCUMENT NUMBER: 142:193054
 TITLE: Novel Cnidarian and Anthozoan chromo/fluoroproteins and cDNA encoding them
 INVENTOR(S): Labas, Yuliia Aleksandrovich; Gurskaya, Nadezda Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey; Matz, Mikhail Vladimirovich
 PATENT ASSIGNEE(S): Russia
 SOURCE: U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of Appl. No. PCT/02US/36499.
 CODEN: USXXCO
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|--|----------|-----------------|----------|
| US 2005032085 | A1 | 20050210 | US 2004-757356 | 20040113 |
| WO 2003042401 | A2 | 20030522 | WO 2002-US36499 | 20021112 |
| WO 2003042401 | A3 | 20031120 | | |
| W: | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | |

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-332980P P 20011113
WO 2002-US36499 A2 20021112

L3 ANSWER 3 OF 5 HCPLUS COPYRIGHT 2006 ACS on STN
 TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
 AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. Claimed sequences were not present at the time of publication.

ACCESSION NUMBER: 2003:511470 HCPLUS
 DOCUMENT NUMBER: 139:65739
 TITLE: Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
 INVENTOR(S): Bevis, Brooke; Glick, Benjamin
 PATENT ASSIGNEE(S): The University of Chicago, USA
 SOURCE: PCT Int. Appl., 65 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 2003054158 | A2 | 20030703 | WO 2002-US40539 | 20021218 |
| WO 2003054158 | A3 | 20031204 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2467383 | AA | 20030703 | CA 2002-2467383 | 20021218 |
| AU 2002357322 | A1 | 20030709 | AU 2002-357322 | 20021218 |
| EP 1456223 | A2 | 20040915 | EP 2002-805620 | 20021218 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK | | | | |
| JP 2006501804 | T2 | 20060119 | JP 2003-554863 | 20021218 |

| | | | | |
|------------------------|----|----------|-----------------|------------|
| US 2005149994 | A1 | 20050707 | US 2004-844064 | 20040511 |
| PRIORITY APPLN. INFO.: | | | US 2001-341723P | P 20011219 |
| | | | WO 2002-US40539 | W 20021218 |

L3 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use
 AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded the same, are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. More specifically, they include GFP of *Heteractis crispa*, *Dendronephthya* sp, *Scolymia cubensis*, *Ricordea florida*, *Montastraea cavernosa*, *Condylactis gigantea*, *Agaricia fragilis*, sequence homolog of *Montrastraea annularis* and RFP of *Zoanthus* sp., *Ricordea florida*, and *Montastraea cavernosa*. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2003:397030 HCAPLUS

DOCUMENT NUMBER: 138:397335

TITLE: cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use

INVENTOR(S): Labas, Yuliia Aleksandrovich; Gurskaya, Nadezda Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey; Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA

SOURCE: PCT Int. Appl., 88 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent
 LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 2003042401 | A2 | 20030522 | WO 2002-US36499 | 20021112 |
| WO 2003042401 | A3 | 20031120 | | |
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| CA 2454031 | AA | 20030522 | CA 2002-2454031 | 20021112 |
| EP 1444245 | A2 | 20040811 | EP 2002-797104 | 20021112 |
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|------------------------|----|----------|-----------------|------------|
| JP 2005509420 | T2 | 20050414 | JP 2003-544215 | 20021112 |
| US 2005032085 | A1 | 20050210 | US 2004-757356 | 20040113 |
| PRIORITY APPLN. INFO.: | | | US 2001-332980P | P 20011113 |
| | | | WO 2002-US36499 | W 20021112 |

L3 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same
 AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded by the same, are provided. The subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific Anthozoan species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp. (NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red" (NFP-6), Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp. "magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2002:978391 HCAPLUS
 DOCUMENT NUMBER: 138:50935
 TITLE: cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same
 INVENTOR(S): Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii A.; Matz, Mikhail V.; Terskikh, Alexey
 PATENT ASSIGNEE(S): Russia
 SOURCE: U.S. Pat. Appl. Publ., 48 pp.; Cont.-in-part of Appl. No. PCT/US00/28477.
 CODEN: USXXCO
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 17
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| US 2002197676 | A1 | 20021226 | US 2001-6922 | 20011204 |
| WO 2000034526 | A1 | 20000615 | WO 1999-US29405 | 19991210 |
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| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| WO 2001027150 | A2 | 20010419 | WO 2000-US28477 | 20001013 |
| WO 2001027150 | A3 | 20011206 | | |
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 WO 2002068459 A2 20020906 WO 2002-US5749 20020220
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 AU 2002254031 A1 20020912 AU 2002-254031 20020220
 US 2003022287 A1 20030130 US 2002-81864 20020220
 US 6969597 B2 20051129
 EP 1385967 A2 20040204 EP 2002-723238 20020220
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 US 2003092884 A1 20030515 US 2002-155809 20020524
 US 2006035330 A1 20060216 US 2005-187622 20050721
 AU 2006200881 A1 20060330 AU 2006-200881 20060301
 PRIORITY APPLN. INFO.:
 US 1999-418529 A2 19991014
 US 1999-418917 B2 19991015
 US 1999-418922 B2 19991015
 US 1999-444338 B2 19991119
 US 1999-444341 B2 19991119
 US 1999-457556 B2 19991209
 US 1999-457898 B2 19991209
 US 1999-458144 B2 19991209
 US 1999-458477 B2 19991209
 WO 1999-US29405 W 19991210
 US 2000-211607P P 20000614
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 US 2000-212070P P 20000614
 WO 2000-US28477 A2 20001013
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FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCPLUS, WPIDS, FSTA, JICST-EPLUS,
 SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006
 E LUKYANOV, S/AU

L1 123 S CNIDARIAN AND (ANTHOZOAN)
L2 11 S L1 AND (NON-BIOLUMINESCENT)
L3 5 S L1 AND (NON-PENNATULACEAN)

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L2 ANSWER 1 OF 11 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Novel nucleic acid encoding interconverted mutant of chromo-or
fluorescent protein which are useful as biosensors, coloring agents;
involving vector-mediated gene transfer and expression in host cell
for use in transgenic plant and transgenic animal construction

AN 2003-22525 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromo-
or fluorescent protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1)
a fragment of a nucleic acid encoding (I); (2) a construct comprising a
vector and a nucleic acid encoding (I); (3) an expression cassette (II)
comprises, a transcriptional initiation region that is functional in an
expression host, a nucleic acid encoding (I) and a transcriptional
termination region functional in the expression host; (4) a cell or its
progeny comprising (II), as part of an extrachromosomal element or
integrated into the genome of a host cell as a result of introduction of
(II) into the host cell; (5) producing a chromo and/or fluorescent
protein, comprises, growing the cell where protein is expressed and
isolating the protein substantially free of other proteins; (6) a protein
(III) or its fragment encoded by the nucleic acid encoding (I) and an
antibody binding specifically to the (III); (7) transgenic cell or its
progeny comprises a transgene which is a nucleic acid encoding (I); (8) a
kit comprising a nucleic acid encoding (I); (9) preparation (M1) of
nucleic acid encoding (I); and (10) a nucleic acid produced by (M1).

BIOTECHNOLOGY - Preparation: Producing a nucleic acid that encodes a
protein having at least one point mutation chosen from positions 148 and
165 as compared to the parent protein produced by the nucleic acid
encoding (I). The produced nucleic acid encodes a protein having point
mutations at both positions 148 and 165. (I) is the fluorescent mutant of
parent non-fluorescent chromoprotein. The nucleic acid produced encodes a
protein further comprising mutations at positions 167 and 203, where (I)
is a non-fluorescent chromoprotein of a parent fluorescent protein
(claimed). Preferred Nucleic Acid: The chromo or fluorescent protein is
from a non-bioluminescent Cnidarian sp and
belongs to Anthozoan sp.. (I) includes a point mutation chosen
from a mutation at positions 148 and 165. (I) is a fluorescent mutant of
a chromoprotein and includes a point mutation at both positions 148 and
165. (I) is a non-fluorescent chromoprotein of a fluorescent protein and
includes a point mutation at positions 167 and 203.

USE - Nucleic acid encoding (I) is useful in any application that
employs a chromo- or fluorescent protein. (III) is useful in any
application that employs a chromo- or fluorescent protein (claimed).
Nucleic acid encoding (I) is useful in the generation of transgenic,
non-human plants or animals or site specific gene modification in cell
lines. Chromoprotein encoded by the nucleic acid is useful as coloring
agents which are capable of imparting color or pigment to a particular
composition of matter e.g. food compositions, pharmaceuticals, cosmetics,
living organisms, etc. The chromoprotein is also useful as labels in
biological analyte detection assays and as selectable markers in
recombinant DNA applications (e.g. the production of transgenic cells and
organisms) and is also useful as sunscreens, selective filters, etc. The
fluorescent protein encoded by the nucleic acid, is useful in
fluorescence resonance energy transfer (FRET) applications and also
useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca²⁺ ion
indicator and as marker of whole cells to detect changes in multicellular
reorganization and migration. The fluorescent proteins are also useful as

second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

EXAMPLE - A purple chromoprotein, asCP from Anemonia sulcata and a red fluorescent protein DsRed from Discosoma Sp. were selected as representatives of chromoprotein (CP) and fluoroprotein (FP) respectively. Site directed and random mutagenesis were performed to transform CP into FP and vice versa. Site directed mutagenesis was performed by PCR with primers containing target substitution using the overlap extension method. All mutants were cloned into pQE30 vector so that recombinant proteins contained 6-histidine tag at their N-termini. Escherichia coli XL1 Blue cells were transformed with the plasmids according to standard protocols and spread onto 3-4 Petri dishes with LB agar media supplemented with ampicillin for selection. After overnight growth at 37degreesC the plates were stored for 2-5 days at room temperature or 4degreesC to allow proteins to mature completely. The plates were washed with phosphate buffered saline (PBS). Cells were disrupted by sonication and soluble recombinant proteins were purified on the TALON metal affinity resin. Absorption spectra of the proteins were recorded on a Beckman DU520 UV/VIS spectrophotometer. The amino acid substitution in asCP mutant in the positions 148 and 165, increased quantum yield of red fluorescence. Serine and valine were substituted at positions 148 and 165 respectively. In fluorescent DsRed, substitutions at position 148, 165, 167 and 203 significantly decreased fluorescence intensity, and the spectral characteristics of these mutants became more close to those of CPs. Non-fluorescent (NF) mutant DsRed-NF carried four amino acid substitutions, specifically. Ser148Cys, Ile165Asn, Lys167Met and Ser203Ala. DsRed-NF possessed a high extinction coefficient and an extremely low quantum yield (less than 0.001). These special characteristics converted DsRed-NF into a true chromoprotein. (56 pages)

ACCESSION NUMBER: 2003-22525 BIOTECHDS

TITLE: Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents;
involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic animal construction

AUTHOR: BULINA M E; CHUDAKOV D; LUKYANOV K A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2003057833 17 Jul 2003

APPLICATION INFO: WO 2002-US41418 23 Dec 2002

PRIORITY INFO: US 2001-343128 26 Dec 2001; US 2001-343128 26 Dec 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-607998 [57]

L2 ANSWER 2 OF 11 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins;

involving vector-mediated gene transfer and expression in Escherichia coli

AN 2003-20948 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or

fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host; (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and (8) a kit comprising (I) or (II).

WIDER DISCLOSURE - (1) homologs of (I); (2) nucleic acids that encode proteins as (I), but differ in sequence of (I) due to degeneracy of genetic code; (3) nucleic acid that hybridize to (I); (4) nucleic acid that encode fusion proteins comprising (VI) and a heterologous protein; (5) homologs or fragments of (VI); and (6) polypeptides that differ from (VI).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) encodes a chromo- or fluorescent mutant of a non-bioluminescent Cnidarian species (e.g. Anthozoan species, preferably Discosoma sp.) chromo- or fluorescent protein. More preferably, (I) encodes a mutant of wild-type Discosoma sp. red fluorescent protein (DsRed), where the nucleic acid encodes a product having a point mutation at at least one of position 2, 5, 6, 21, 41, 42, 44 and 117 relative to wild-type DsRed, preferably a product having a point mutation at at least one of position 145 and 217. The nucleic acid encoding the product has a sequence of residues that is substantially the same or identical to a nucleotide sequence of at least 10 residues in length of a fully defined wild-type DsRed nucleic acid sequence (S1) as given in the specification.

USE - (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed). (I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used in assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

EXAMPLE - Wild-type Discosoma sp. red fluorescent protein (DsRed), an orange-red fluorescence with an emission maximum at 583 nm, had several problems for use as a fluorescent reporter, e.g., slow maturation. Therefore, to identify rapidly maturing DsRed variants, an earlier method for visualizing green fluorescent protein (GFP) fluorescence in microbial colonies was modified. Hexahistidine-tagged DsRed was produced at high levels in Escherichia coli. The fluorescence

of the bacterial colonies was excited by placing a 520 +/- 20 nm bandpass filter over the lens of a slide projector, and the emission was detected. A library of mutant expression plasmids was generated using error-prone PCR to amplify the DsRed1 template. This library was transformed into E. coli, and over 100000 transformant colonies were examined. Colonies producing the wild-type DsRed1 protein required two days to develop significant fluorescence, but three mutant colonies showed strong fluorescence after one day of growth. Sequencing revealed that the three mutant plasmids were distinct, but that all of them contained an N42H codon change. Therefore a variant was generated that had only the N42H substitution. The N42H variant was purified in parallel with DsRed1, and the two proteins were analyzed by spectrofluorometry. The spectra of purified DsRed1 changed over a period of days as the protein matured. By contrast, the spectra of the purified N42H variant remained stable over time consistent with rapid maturation. In addition to accelerating maturation, the N42H substitution altered the spectral properties of the mature protein. Mature DsRed1 was assumed to be an equilibrium mixture of red fluorescent molecules and some green fluorescent molecules that were spectrally similar to GFP. The GFP-like species had a blue excitation peak at approximately 480 nm and a green emission peak at approximately 500 nm, but DsRed was tetramer, so excitation of the green molecules often resulted in the fluorescence resonance energy transfer (FRET) with neighboring red molecules to produce and emission. The FRET effect, together with the relatively low percentage of green molecules in mature DsRed1, yielded a very small peak of green emission relative to the red emission. In the N42H variant, the peaks of blue excitation and green emission were dramatically enhanced, indicating that the equilibrium had shifted so that a target percentage of the mature molecules contained the green chromophore. Because the N42H substitution considerably increased the size of the side chain, a more conservative N42Q substitution was also tried. This mutation required two base changes and probably would not have been present in the original mutant collection. The N42Q variant retained the rapid maturation property of the N42H variant, but showed much less blue excitation and green emission. The N42Q variant was therefore chosen as the starting point for further study. Additional mutagenesis yielded DsRed variants that showed even faster maturation and lower green emission than the original N42Q variant. After six rounds of mutagenesis, three optimized variants were selected and termed DsRed T1, DsRed T3 and DsRed T4. The spectral properties of DsRed T4 were virtually identical to those of DsRed T1 and very similar to those of the wild-type DsRed1. Compared with DsRed T1 and DsRed T4, DsRed T3 was somewhat brighter but has a significantly higher peak of blue excitation and a marginally higher peak of green emission. The optimized DsRed variants were examined both in vivo and in vitro. As judged by colony fluorescence, colony size, and plasmid stability, these variants were less toxic to E. coli than DsRed1, and they developed fluorescence more efficiently at growth temperatures of 37 degreesC and higher. Like wild-type DsRed, the optimized variants appeared to be tetrameric, they exhibited FRET between the green and red molecules. The end result was pair of optimized variants termed DsRed T3 and DsRed T4. DsRed T4, DsRed T3 matured rapidly, and the purified protein was nearly as bright as mature wild-type DsRed. Making this variant well suited to single-color imaging of red fluorescence. DsRed T3 had a higher peak of blue excitation and a slightly higher peak of green emission than wild-type DsRed. DsRed T4 had fluorescence spectra very similar to those of wild-type DsRed and yielded negligible contamination of the GFP signal. Although purified DsRed T4 was only about half as bright as DsRed T3, this effect was partially offset in vivo because DsRed T4 matures nearly twice as fast as DsRed T3. Thus, DsRed T4 was probably the best variant for most applications. (65 pages)

ACCESSION NUMBER: 2003-20948 BIOTECHDS

TITLE: Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or

fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins;
involving vector-mediated gene transfer and expression in *Escherichia coli*

AUTHOR: BEVIS B; GLICK B
PATENT ASSIGNEE: UNIV CHICAGO
PATENT INFO: WO 2003054158 3 Jul 2003
APPLICATION INFO: WO 2002-US40539 18 Dec 2002
PRIORITY INFO: US 2001-341723 19 Dec 2001; US 2001-341723 19 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-569236 [53]

L2 ANSWER 3 OF 11 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

AN 2003-09318 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host; (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I); (8) a transgenic organism (IX) comprising a transgene that comprises (I); (9) production of (VI); (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent; (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

WIDER DISCLOSURE - Also disclosed are: (1) a composition comprising (I), (V), (VI), (VII) or recombinant vectors; (2) homologs of (I) or (VI); (3) nucleic acids that encode the proteins encoded by (I), but differ in sequence from (I) due to the degeneracy of the genetic code; (4) nucleic acids that hybridize to (I) under stringent conditions; (5) nucleic acids encoding variants e.g., mutants of (I); and (6) nucleic acids that encode fusion proteins of (I) or its fragments.

BIOTECHNOLOGY - Preparation: (VI) is produced by growing (V), where (VI) is expressed, and isolating (VI) that is substantially free of other proteins (claimed). Preferred Nucleic Acid: In (I), the kindling stimulus is light of a kindling wavelength, intensity and duration effective to kindle the kindling fluorescent protein. The kindling wavelength of the kindling stimulus ranges from about 200-1500 nm, the kindling intensity of the kindling stimulus ranges from 0.01-106 W/cm², and the kindling duration of the kindling stimulus ranges from 1 millisecond to about 60 minutes. The kindling fluorescent protein does not have a sequence that is identical to a sequence comprising 232 or 215 amino acids fully defined in the specification. The kindling fluorescent protein is a mutant of a wild type kindling fluorescent protein. The kindling

fluorescent protein is a wild type protein or its mutant from a non-bioluminescent Cnidarian species, preferably an Anthozoan species. The second state is transient. The second fluorescent state is permanent. (I) is an isolated nucleic acid. Preferred Method: In (M), the kindling fluorescent protein is present inside an organism or cell.

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed). (I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

EXAMPLE - Generation of and initial characterization of kindling fluorescent proteins was as follows. Routine target i.e. site specific, and random mutagenesis of wild type asFP595 and *Heteractis crispa* chromoproteins was carried out. The asFP595 (asCP) had a sequence comprising 232 amino acids fully defined in the specification and was encoded by a nucleotide coding sequence comprising 767 base pairs fully defined in the specification. The *H.crispa* chromoprotein had a sequence comprising 215 amino acids fully defined in the specification was encoded by a nucleotide coding sequence comprising 760 base pairs fully defined in the specification. Mutagenesis was performed by the overlap extension method. Briefly, two overlapping fragments of each FP coding region were amplified. Polymerase chain reaction (PCR) was carried out using Advantage 2 polymerase mix in 1xmanufacturer's buffer supplemented with 100 μM of each dNTP, 0.2 μM of each primer, and 1 ng of plasmid DNA in 25 μl (final volume). To remove plasmids encoding wild type proteins, the 5' and 3'-fragments were excised from 2% low-melting agarose gel in 1xTAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. For each particular mutant, appropriate 5'- and 3'-fragments were combined to obtain full-length cDNA as follows. Equal volumes of 5'-fragment solution, 3'-fragment solution and 3xPCR mixture containing Advantage 2 polymerase mix, buffer and dNTPs were mixed together and subjected to 2-3 cycles of 95°C for 20 s, 65°C for 30 min, 72°C for 30 s. Then, the reaction was diluted 10 fold and 1 μl of the diluted sample was used as a template for PCR with forward and reverse cloning primers. As a result, ready-for-cloning fragments containing full-length coding regions with target substitution(s) were generated. Mutant PC products were digested with endonucleases, for which the cloning primers contained sites, and then cloned into pQE30, digested with endonucleases generating complementary overhangs. Each of the recombinant proteins generated by both cloning-expression systems contained a 6xHis tag on the N- or C-terminus. Selected *Escherichia coli* clones were grown at 37°C in 50 ml to an optical density of (OD) 0.6. At that point, the expression of recombinant FP was induced with 0.2 mM isopropyl-beta-D-thiogalactoside (IPTG). The cultures were then incubated overnight. The following day, cells were harvested by centrifugation, resuspended in buffer and disrupted by sonication. Fluorescent proteins were purified from the soluble fraction.

Proteins were at least 95% pure according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (96 pages)

ACCESSION NUMBER: 2003-09318 BIOTECHDS

TITLE: Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

AUTHOR: LUKYANOV S A; CHUDAKOV D; LUKYANOV K

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2002096924 5 Dec 2002

APPLICATION INFO: WO 2002-US16379 24 May 2002

PRIORITY INFO: US 2001-329176 11 Oct 2001; US 2001-293752 25 May 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-156788 [15]

L2 ANSWER 4 OF 11 HCPLUS COPYRIGHT 2006 ACS on STN

TI Rapidly maturing fluorescent proteins and methods for using the same

AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. red fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:588435 HCPLUS

DOCUMENT NUMBER: 143:112117

TITLE: Rapidly maturing fluorescent proteins and methods for using the same

INVENTOR(S): Bevis, Brooke; Glick, Benjamin

PATENT ASSIGNEE(S): The University of Chicago, USA

SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of Appl. No. PCT/US02/40539.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|------|----------|-----------------|----------|
| US 2005149994 | A1 | 20050707 | US 2004-844064 | 20040511 |
| WO 2003054158 | A2 | 20030703 | WO 2002-US40539 | 20021218 |
| WO 2003054158 | A3 | 20031204 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,

PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,
 UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
 KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
 FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ,
 CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-341723P P 20011219
 WO 2002-US40539 A2 20021218

L2 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI Novel Cnidarian and Anthozoan chromo/fluoroproteins
 and cDNA encoding them
 AB DNA encoding novel chromo/fluoroproteins as well as the encoded proteins
 are provided. The proteins of interest are proteins that are colored
 and/or fluorescent, where this feature arises from the interaction of two
 or more residues of the protein. The subject proteins are further
 characterized in that they are either obtained from non-
 bioluminescent Cnidarian, e.g., Anthozoan,
 species or are obtained from Anthozoan non- Pennatulacean (sea
 pen) species. Specific proteins of interest include the following
 specific proteins: Heteractis crispa hcriGFP; Dendronephthya dendGFP;
 Zoanthus zoanRFP; Scolymia cubensis scubGFP1 and scubGFP2; Ricordea
 florida rfloRFP, rfloGFP2, and rfloGFP; Montastraea cavernosa mcavRFP,
 mcavGFP, and mcavGFP2; Condylactis gigantea cgigGFP; Agaricia fragilis
 afraGFP; and Montastraea annularis mannFP. Also of interest are proteins
 that are substantially similar to, or mutants of, the above specific
 proteins. Also provided are fragments of the nucleic acids and the
 peptides encoded thereby, as well as antibodies to the subject proteins
 and transgenic cells and organisms. The subject protein and nucleic acid
 compns. find use in a variety of different applications. Finally, kits
 for use in such applications, e.g., that include the subject nucleic acid
 compns., are provided.

ACCESSION NUMBER: 2005:122690 HCAPLUS

DOCUMENT NUMBER: 142:193054

TITLE: Novel Cnidarian and Anthozoan
chromo/fluoroproteins and cDNA encoding them

INVENTOR(S): Labas, Yulii Aleksandrovich; Gurskaya, Nadezda
Georgievna; Yanushevich, Yuriy; Fradkov, Arcady
Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;
Matz, Mikhail Vladimirovich

PATENT ASSIGNEE(S): Russia

SOURCE: U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of Appl.
No. PCT/02US/36499.

CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| US 2005032085 | A1 | 20050210 | US 2004-757356 | 20040113 |
| WO 2003042401 | A2 | 20030522 | WO 2002-US36499 | 20021112 |
| WO 2003042401 | A3 | 20031120 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT,
TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, | | | | |

CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
 PRIORITY APPLN. INFO.: US 2001-332980P P 20011113
 WO 2002-US36499 A2 20021112

L2 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
 AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. Claimed sequences were not present at the time of publication.

ACCESSION NUMBER: 2003:511470 HCAPLUS
 DOCUMENT NUMBER: 139:65739
 TITLE: Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
 INVENTOR(S): Bevis, Brooke; Glick, Benjamin
 PATENT ASSIGNEE(S): The University of Chicago, USA
 SOURCE: PCT Int. Appl., 65 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|------------|
| WO 2003054158 | A2 | 20030703 | WO 2002-US40539 | 20021218 |
| WO 2003054158 | A3 | 20031204 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2467383 | AA | 20030703 | CA 2002-2467383 | 20021218 |
| AU 2002357322 | A1 | 20030709 | AU 2002-357322 | 20021218 |
| EP 1456223 | A2 | 20040915 | EP 2002-805620 | 20021218 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK | | | | |
| JP 2006501804 | T2 | 20060119 | JP 2003-554863 | 20021218 |
| US 2005149994 | A1 | 20050707 | US 2004-844064 | 20040511 |
| PRIORITY APPLN. INFO.: | | | US 2001-341723P | P 20011219 |
| | | | WO 2002-US40539 | W 20021218 |

L2 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use
 AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded the same, are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. More specifically, they include GFP of *Heteractis crispa*, *Dendronephthya* sp, *Scolymia cubensis*, *Ricordea florida*, *Montastraea cavernosa*, *Condylactis gigantea*, *Agaricia fragilis*, sequence homolog of *Montrastraea annularis* and RFP of *Zoanthus* sp., *Ricordea florida*, and *Montastraea cavernosa*. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2003:397030 HCAPLUS
 DOCUMENT NUMBER: 138:397335
 TITLE: cDNAs encoding chromo/fluoroproteins from non-bioluminescent Cnidarian species or non-Pennatulacean (sea pen) species and their use
 INVENTOR(S): Labas, Yulii Aleksandrovich; Gurskaya, Nadezda Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey; Matz, Mikhail Vladimirovich
 PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA
 SOURCE: PCT Int. Appl., 88 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|------------|
| WO 2003042401 | A2 | 20030522 | WO 2002-US36499 | 20021112 |
| WO 2003042401 | A3 | 20031120 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2454031 | AA | 20030522 | CA 2002-2454031 | 20021112 |
| EP 1444245 | A2 | 20040811 | EP 2002-797104 | 20021112 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK | | | | |
| JP 2005509420 | T2 | 20050414 | JP 2003-544215 | 20021112 |
| US 2005032085 | A1 | 20050210 | US 2004-757356 | 20040113 |
| PRIORITY APPLN. INFO.: | | | US 2001-332980P | P 20011113 |
| | | | WO 2002-US36499 | W 20021112 |

L2 ANSWER 8 OF 11 HCAPLUS COPYRIGHT 2006 ACS on STN
TI cDNA and protein sequences of novel chromo/fluoroproteins from non
-bioluminescent Cnidarian species or are obtained from
non-Pennatulacean (sea pen) species and methods for using the same
AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants
thereof, as well as the proteins encoded by the same, are provided. The
subject proteins of interest are proteins that are colored and/or
fluorescent, where this feature arises from the interaction of two or more
residues of the protein. The subject proteins are further characterized
in that they are either obtained from non-bioluminescent
Cnidarian, e.g., Anthozoan, species or are obtained from
non-Pennatulacean (sea pen) species. Specific proteins of interest
include proteins obtained from the following specific Anthozoan
species: Anemonia majano (NFP-1), Clavularia sp. (NFP-2), Zoanthus sp.
(NFP-3 & NFP-4), Discosoma striata (NFP-5), Discosoma sp. "red" (NFP-6),
Anemonia sulcata (NFP-7), Discosoma sp "green" (NFP-8), and Discosoma sp.
"magenta" (NFP-9). Also of interest are proteins that are substantially
similar to, or mutants of, the above specific proteins. Also provided are
fragments of the nucleic acids and the peptides encoded thereby, as well
as antibodies to the subject proteins and transgenic cells and organisms.
The subject protein and nucleic acid compns. find use in a variety of
different applications. Finally, kits for use in such applications, e.g.,
that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2002:978391 HCAPLUS
DOCUMENT NUMBER: 138:50935
TITLE: cDNA and protein sequences of novel
chromo/fluoroproteins from non-
bioluminescent Cnidarian species or
are obtained from non-Pennatulacean (sea pen) species
and methods for using the same
INVENTOR(S): Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii
A.; Matz, Mikhail V.; Terskikh, Alexey
PATENT ASSIGNEE(S): Russia
SOURCE: U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of Appl.
No. PCT/US00/28477.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 17
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| US 2002197676 | A1 | 20021226 | US 2001-6922 | 20011204 |
| WO 2000034526 | A1 | 20000615 | WO 1999-US29405 | 19991210 |
| W: JP | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE | | | | |
| WO 2001027150 | A2 | 20010419 | WO 2000-US28477 | 20001013 |
| WO 2001027150 | A3 | 20011206 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU,
SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN,
YU, ZA, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY,
DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ,
CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2434737 | AA | 20020906 | CA 2002-2434737 | 20020220 |
| WO 2002068459 | A2 | 20020906 | WO 2002-US5749 | 20020220 |
| WO 2002068459 | A3 | 20031127 | | |

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
 PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ,
 UA, UG, US, UZ, VN, YU, ZA, ZM, ZW
 RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
 KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB,
 GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA,
 GN, GQ, GW, ML, MR, NE, SN, TD, TG
 AU 2002254031 A1 20020912 AU 2002-254031 20020220
 US 2003022287 A1 20030130 US 2002-81864 20020220
 US 6969597 B2 20051129
 EP 1385967 A2 20040204 EP 2002-723238 20020220
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO, MK, CY, AL, TR
 JP 2004536571 T2 20041209 JP 2002-567969 20020220
 US 2003092884 A1 20030515 US 2002-155809 20020524
 US 2006035330 A1 20060216 US 2005-187622 20050721
 AU 2006200881 A1 20060330 AU 2006-200881 20060301
 PRIORITY APPLN. INFO.:
 US 1999-418529 A2 19991014
 US 1999-418917 B2 19991015
 US 1999-418922 B2 19991015
 US 1999-444338 B2 19991119
 US 1999-444341 B2 19991119
 US 1999-457556 B2 19991209
 US 1999-457898 B2 19991209
 US 1999-458144 B2 19991209
 US 1999-458477 B2 19991209
 WO 1999-US29405 W 19991210
 US 2000-211607P P 20000614
 US 2000-211609P P 20000614
 US 2000-211626P P 20000614
 US 2000-211627P P 20000614
 US 2000-211687P P 20000614
 US 2000-211766P P 20000614
 US 2000-211880P P 20000614
 US 2000-211888P P 20000614
 US 2000-212070P P 20000614
 WO 2000-US28477 A2 20001013
 US 1998-210330 A 19981211
 AU 2001-10867 A3 20001013
 US 2001-270983P P 20010221
 US 2001-293752P P 20010525
 US 2001-329176P P 20011011
 US 2001-976673 A 20011012
 US 2001-6922 A 20011204
 US 2002-81864 A1 20020220
 WO 2002-US5749 W 20020220

L2 ANSWER 9 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
 TI Nové́l nucleic acid encoding interconverted mutant of chromo- or fluorescent
 protein which are useful as biosensors, coloring agents.
 AN 2003-607998 [57] WPIDS
 AB WO2003057833 A UPAB: 20030906
 NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromo-
 or fluorescent protein, is new.
 DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
 (1) a fragment of a nucleic acid encoding (I);
 (2) a construct comprising a vector and a nucleic acid encoding (I);
 (3) an expression cassette (II) comprises, a transcriptional
 initiation region that is functional in an expression host, a nucleic acid
 encoding (I) and a transcriptional termination region functional in the

expression host;

(4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell;

(5) producing a chromo and/or fluorescent protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins;

(6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III);

(7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I);

(8) a kit comprising a nucleic acid encoding (I);

(9) preparation (M1) of nucleic acid encoding (I); and

(10) a nucleic acid produced by (M1).

USE - Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. Chromoprotein encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The chromoprotein is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca²⁺ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

DESCRIPTION OF DRAWING(S) - The figure shows the normalized spectra for selected mutants of asCP and DsRed.

Dwg.3/3

ACCESSION NUMBER: 2003-607998 [57] WPIDS

DOC. NO. CPI: C2003-165725

TITLE: Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.

DERWENT CLASS: B04 D16

INVENTOR(S): BULINA, M E; CHUDAKOV, D; LUKYANOV, K A

PATENT ASSIGNEE(S): (CLON-N) CLONTECH LAB INC; (BULI-I) BULINA M E; (CHUD-I) CHUDAKOV D; (LUKY-I) LUKYANOV K A

COUNTRY COUNT: 103

PATENT INFORMATION:

| PATENT NO | KIND DATE | WEEK | LA | PG |
|-----------|-----------|------|----|----|
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|---------------|--------------------------|----|-------|-------|
| WO 2003057833 | A2 20030717 (200357)* EN | 56 | ----- | ----- |
|---------------|--------------------------|----|-------|-------|

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|--|-------|-------|-------|-------|
| RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW | ----- | ----- | ----- | ----- |
|--|-------|-------|-------|-------|

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|--|-------|-------|-------|-------|
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT | ----- | ----- | ----- | ----- |
|--|-------|-------|-------|-------|

RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
 ZM ZW
 AU 2002367391 A1 20030724 (200421)
 US 2004248180 A1 20041209 (200481)
 EP 1504017 A2 20050209 (200512) EN
 R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC
 MK NL PT RO SE SI SK TR
 JP 2005514032 W 20050519 (200538) 38

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|--------------------------|--|----------------------------------|
| WO 2003057833 | A2 | WO 2002-US41418 | 20021223 |
| AU 2002367391 | A1 | AU 2002-367391 | 20021223 |
| US 2004248180 | A1 Provisional
CIP of | US 2001-343128P
WO 2002-US41418
US 2004-845484 | 20011226
20021223
20040512 |
| EP 1504017 | A2 | EP 2002-806227
WO 2002-US41418 | 20021223
20021223 |
| JP 2005514032 | W | WO 2002-US41418
JP 2003-558135 | 20021223
20021223 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---------------|-------------|---------------|
| AU 2002367391 | A1 Based on | WO 2003057833 |
| EP 1504017 | A2 Based on | WO 2003057833 |
| JP 2005514032 | W Based on | WO 2003057833 |

PRIORITY APPLN. INFO: US 2001-343128P 20011226; US
2004-845484 20040512

L2 ANSWER 10 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
 TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent
 mutant of a Cnidarian chromo- or fluorescent protein or its
 mutant, useful for applications involving chromo- or fluorescent proteins.
 AN 2003-569236 [53] WPIDS
 AB WO2003054158 A UPAB: 20030820
 NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or
 fluorescent mutant of a Cnidarian chromo- or fluorescent protein
 or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host;
- (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell;
- (5) a protein (VI) or its fragment encoded by (I);
- (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and
- (8) a kit comprising (I) or (II).

USE - (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed).

(I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used in assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

Dwg.0/4

ACCESSION NUMBER: 2003-569236 [53] WPIDS
 DOC. NO. CPI: C2003-153632
 TITLE: Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins.
 DERWENT CLASS: B04 D16
 INVENTOR(S): BEVIS, B; GLICK, B
 PATENT ASSIGNEE(S): (UYCH-N) UNIV CHICAGO
 COUNTRY COUNT: 103
 PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---------------|---|--------------------|------|----|----|
| ----- | | | | | |
| WO 2003054158 | A2 | 20030703 (200353)* | EN | 65 | |
| RW: | AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW | | | | |
| W: | AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW | | | | |
| AU 2002357322 | A1 | 20030709 (200428) | | | |
| EP 1456223 | A2 | 20040915 (200460) | EN | | |
| R: | AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR | | | | |
| US 2005149994 | A1 | 20050707 (200547) | | | |
| JP 2006501804 | W | 20060119 (200606) | | 43 | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|--------------------------|------------------------------------|----------------------|
| ----- | | | |
| WO 2003054158 | A2 | WO 2002-US40539 | 20021218 |
| AU 2002357322 | A1 | AU 2002-357322 | 20021218 |
| EP 1456223 | A2 | EP 2002-805620 | 20021218 |
| | | WO 2002-US40539 | 20021218 |
| US 2005149994 | A1 Provisional
CIP of | US 2001-341723P
WO 2002-US40539 | 20011219
20021218 |
| | | US 2004-844064 | 20040511 |
| JP 2006501804 | W | WO 2002-US40539
JP 2003-554863 | 20021218
20021218 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---------------|-------------|---------------|
| AU 2002357322 | A1 Based on | WO 2003054158 |
| EP 1456223 | A2 Based on | WO 2003054158 |
| JP 2006501804 | W Based on | WO 2003054158 |

PRIORITY APPLN. INFO: US 2001-341723P 20011219; US
 2004-844064 20040511

L2 ANSWER 11 OF 11 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
 TI Novel nucleic acid that is present in other than its natural environment
 and that encodes kindling fluorescent protein, is useful in labeling
 protocols, e.g. labeling proteins, organelles, cells and organisms.

AN 2003-156788 [15] WPIDS

AB WO 200296924 A UPAB: 20030303

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host;
- (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell;
- (5) a protein (VI) or its fragment encoded by (I);
- (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I);
- (8) a transgenic organism (IX) comprising a transgene that comprises (I);
- (9) production of (VI);
- (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent;
- (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and
- (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed).

(I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

Dwg.0/10
 ACCESSION NUMBER: 2003-156788 [15] WPIDS
 DOC. NO. CPI: C2003-040710
 TITLE: Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms.
 DERWENT CLASS: B04 D16
 INVENTOR(S): CHUDAKOV, D; LUKYANOV, K; LUKYANOV, S A
 PATENT ASSIGNEE(S): (CHUD-I) CHUDAKOV D; (LUKY-I) LUKYANOV K; (LUKY-I) LUKYANOV S A; (CLON-N) CLONTECH LAB INC
 COUNTRY COUNT: 101
 PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---------------|--|--------------------|------|----|----|
| WO 2002096924 | A1 | 20021205 (200315)* | EN | 96 | |
| RW: | AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW | | | | |
| W: | AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW | | | | |
| US 2003092884 | A1 | 20030515 (200335) | | | |
| EP 1390379 | A1 | 20040225 (200415) | EN | | |
| R: | AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR | | | | |
| AU 2002316164 | A1 | 20021209 (200452) | | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|-------------|-----------------|----------|
| WO 2002096924 | A1 | WO 2002-US16379 | 20020524 |
| US 2003092884 | Provisional | US 2001-293752P | 20010525 |
| | Provisional | US 2001-329176P | 20011011 |
| | | US 2002-155809 | 20020524 |
| EP 1390379 | A1 | EP 2002-746443 | 20020524 |
| | | WO 2002-US16379 | 20020524 |
| AU 2002316164 | A1 | AU 2002-316164 | 20020524 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---------------|-------------|---------------|
| EP 1390379 | A1 Based on | WO 2002096924 |
| AU 2002316164 | A1 Based on | WO 2002096924 |

PRIORITY APPLN. INFO: US 2001-329176P 20011011; US
 2001-293752P 20010525; US
 2002-155809 20020524

=> d his

(FILE 'HOME' ENTERED AT 11:08:06 ON 22 SEP 2006)

FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCPLUS, WPIDS, FSTA, JICST-EPLUS,
 SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006
 E LUKYANOV, S/AU

L1 123 S CNIDARIAN AND (ANTHOZOAN)
 L2 11 S L1 AND (NON-BIOLUMINESCENT)

L3 5 S L1 AND (NON-PENNATULACEAN)

=> s 11 and nucleic acid
4 FILES SEARCHED...

L4 16 L1 AND NUCLEIC ACID

=> d 114 ti abs ibib tot

L14 NOT FOUND

The L-number entered has not been defined in this session, or it has been deleted. To see the L-numbers currently defined in this session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> s 14 ti abs ibib tot

MISSING OPERATOR L4 TI

The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> d his

(FILE 'HOME' ENTERED AT 11:08:06 ON 22 SEP 2006)

FILE 'MEDLINE, BIOSIS, BIOTECHDS, HCPLUS, WPIDS, FSTA, JICST-EPLUS, SCISEARCH' ENTERED AT 11:09:40 ON 22 SEP 2006

E LUKYANOV, S/AU

L1 123 S CNIDARIAN AND (ANTHOZOAN)
L2 11 S L1 AND (NON-BIOLUMINESCENT)
L3 5 S L1 AND (NON-PENNATULACEAN)
L4 16 S L1 AND NUCLEIC ACID

=> d 14 ti abs ibib tot

L4 ANSWER 1 OF 16 MEDLINE on STN

TI The evolution of nuclear receptors: evidence from the coral Acropora.
AB We have amplified and sequenced PCR products derived from 10 nuclear receptor (NR) genes from the anthozoan cnidarian Acropora millepora, including five products corresponding to genes not previously reported from the phylum Cnidaria. cDNAs corresponding to seven of these products were sequenced and at least three encode full-length proteins, increasing the number of complete cnidarian NR coding sequences from one to four. All clear orthologs of Acropora NRs either lack an activation domain or lack a known ligand, consistent with the idea that the ancestral nuclear receptor was without a ligand. Phylogenetic analyses indicate that most, and possibly all, presently identified cnidarian NRs are members of NR subfamily 2, suggesting that the common ancestor of all known nuclear receptors most resembled members of this subfamily.

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ACCESSION NUMBER: 2001558273 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11603940

TITLE: The evolution of nuclear receptors: evidence from the coral Acropora.

AUTHOR: Grasso L C; Hayward D C; Trueman J W; Hardie K M; Janssens P A; Ball E E

CORPORATE SOURCE: Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia.

SOURCE: Molecular phylogenetics and evolution, (2001 Oct) Vol. 21, No. 1, pp. 93-102.

Journal code: 9304400. ISSN: 1055-7903.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200112

ENTRY DATE: Entered STN: 18 Oct 2001
 Last Updated on STN: 22 Jan 2002
 Entered Medline: 5 Dec 2001

L4 ANSWER 2 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI Novel nucleic acid encoding interconverted mutant of
 chromo- or fluorescent protein which are useful as biosensors, coloring
 agents;
 involving vector-mediated gene transfer and expression in host cell
 for use in transgenic plant and transgenic animal construction
AN 2003-22525 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Nucleic acid encoding an interconverted
mutant (I) of a chromo- or fluorescent protein, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1)
a fragment of a nucleic acid encoding (I); (2) a
construct comprising a vector and a nucleic acid
encoding (I); (3) an expression cassette (II) comprises, a
transcriptional initiation region that is functional in an expression
host, a nucleic acid encoding (I) and a
transcriptional termination region functional in the expression host; (4)
a cell or its progeny comprising (II), as part of an extrachromosomal
element or integrated into the genome of a host cell as a result of
introduction of (II) into the host cell; (5) producing a chromo and/or
fluorescent protein, comprises, growing the cell where protein is
expressed and isolating the protein substantially free of other proteins;
(6) a protein (III) or its fragment encoded by the nucleic
acid encoding (I) and an antibody binding specifically to the
(III); (7) transgenic cell or its progeny comprises a transgene which is
a nucleic acid encoding (I); (8) a kit comprising a
nucleic acid encoding (I); (9) preparation (M1) of
nucleic acid encoding (I); and (10) a nucleic
acid produced by (M1).

BIOTECHNOLOGY - Preparation: Producing a nucleic
acid that encodes a protein having at least one point mutation
chosen from positions 148 and 165 as compared to the parent protein
produced by the nucleic acid encoding (I). The
produced nucleic acid encodes a protein having point
mutations at both positions 148 and 165. (I) is the fluorescent mutant of
parent non-fluorescent chromoprotein. The nucleic acid
produced encodes a protein further comprising mutations at positions 167
and 203, where (I) is a non-fluorescent chromoprotein of a parent
fluorescent protein (claimed). Preferred Nucleic Acid
: The chromo or fluorescent protein is from a non-bioluminescent
Cnidarian sp and belongs to Anthozoan sp.. (I) includes
a point mutation chosen from a mutation at positions 148 and 165. (I) is
a fluorescent mutant of a chromoprotein and includes a point mutation at
both positions 148 and 165. (I) is a non-fluorescent chromoprotein of a
fluorescent protein and includes a point mutation at positions 167 and
203.

USE - Nucleic acid encoding (I) is useful in any
application that employs a chromo- or fluorescent protein. (III) is
useful in any application that employs a chromo- or fluorescent protein
(claimed). Nucleic acid encoding (I) is useful in the
generation of transgenic, non-human plants or animals or site specific
gene modification in cell lines. Chromoprotein encoded by the
nucleic acid is useful as coloring agents which are
capable of imparting color or pigment to a particular composition of
matter e.g. food compositions, pharmaceuticals, cosmetics, living
organisms, etc. The chromoprotein is also useful as labels in biological
analyte detection assays and as selectable markers in recombinant DNA
applications (e.g. the production of transgenic cells and organisms) and
is also useful as sunscreens, selective filters, etc. The fluorescent

protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca²⁺ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

EXAMPLE - A purple chromoprotein, asCP from Anemonia sulcata and a red fluorescent protein DsRed from Discosoma Sp. were selected as representatives of chromoprotein (CP) and fluoroprotein (FP) respectively. Site directed and random mutagenesis were performed to transform CP into FP and vice versa. Site directed mutagenesis was performed by PCR with primers containing target substitution using the overlap extension method. All mutants were cloned into pQE30 vector so that recombinant proteins contained 6-histidine tag at their N-termini. Escherichia coli XL1 Blue cells were transformed with the plasmids according to standard protocols and spread onto 3-4 Petri dishes with LB agar media supplemented with ampicillin for selection. After overnight growth at 37degreesC the plates were stored for 2-5 days at room temperature or 4degreesC to allow proteins to mature completely. The plates were washed with phosphate buffered saline (PBS). Cells were disrupted by sonication and soluble recombinant proteins were purified on the TALON metal affinity resin. Absorption spectra of the proteins were recorded on a Beckman DU520 UV/VIS spectrophotometer. The amino acid substitution in asCP mutant in the positions 148 and 165, increased quantum yield of red fluorescence. Serine and valine were substituted at positions 148 and 165 respectively. In fluorescent DsRed, substitutions at position 148, 165, 167 and 203 significantly decreased fluorescence intensity, and the spectral characteristics of these mutants became more close to those of CPs. Non-fluorescent (NF) mutant DsRed-NF carried four amino acid substitutions, specifically. Ser148Cys, Ile165Asn, Lys167Met and Ser203Ala. DsRed-NF possessed a high extinction coefficient and an extremely low quantum yield (less than 0.001). These special characteristics converted DsRed-NF into a true chromoprotein. (56 pages)

ACCESSION NUMBER: 2003-22525 BIOTECHDS

TITLE: Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors; coloring agents; involving vector-mediated gene transfer and expression in host cell for use in transgenic plant and transgenic animal construction

AUTHOR: BULINA M E; CHUDAKOV D; LUKYANOV K A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2003057833 17 Jul 2003

APPLICATION INFO: WO 2002-US41418 23 Dec 2002

PRIORITY INFO: US 2001-343128 26 Dec 2001; US 2001-343128 26 Dec 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-607998 [57]

L4 ANSWER 3 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins;

involving vector-mediated gene transfer and expression in Escherichia coli
AN 2003-20948 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host; (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and (8) a kit comprising (I) or (II).

WIDER DISCLOSURE - (1) homologs of (I); (2) nucleic acids that encode proteins as (I), but differ in sequence of (I) due to degeneracy of genetic code; (3) nucleic acid that hybridize to (I); (4) nucleic acid that encode fusion proteins comprising (VI) and a heterologous protein; (5) homologs or fragments of (VI); and (6) polypeptides that differ from (VI).

BIOTECHNOLOGY - Preferred Nucleic Acid: (I) encodes a chromo- or fluorescent mutant of a non-bioluminescent Cnidarian species (e.g. Anthozoan species, preferably Discosoma sp.) chromo- or fluorescent protein. More preferably, (I) encodes a mutant of wild-type Discosoma sp. red fluorescent protein (DsRed), where the nucleic acid encodes a product having a point mutation at at least one of position 2, 5, 6, 21, 41, 42, 44 and 117 relative to wild-type DsRed, preferably a product having a point mutation at at least one of position 145 and 217. The nucleic acid encoding the product has a sequence of residues that is substantially the same or identical to a nucleotide sequence of at least 10 residues in length of a fully defined wild-type DsRed nucleic acid sequence (S1) as given in the specification.

USE - (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed). (I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used in assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

EXAMPLE - Wild-type *Discosoma* sp. red fluorescent protein (DsRed), an orange-red fluorescence with an emission maximum at 583 nm, had several problems for use as a fluorescent reporter, e.g., slow maturation. Therefore, to identify rapidly maturing DsRed variants, an earlier method for visualizing green fluorescent protein (GFP) fluorescence in microbial colonies was modified. Hexahistidine-tagged DsRed was produced at high levels in *Escherichia coli*. The fluorescence of the bacterial colonies was excited by placing a 520 +/- 20 nm bandpass filter over the lens of a slide projector, and the emission was detected. A library of mutant expression plasmids was generated using error-prone PCR to amplify the DsRed1 template. This library was transformed into *E. coli*, and over 100000 transformant colonies were examined. Colonies producing the wild-type DsRed1 protein required two days to develop significant fluorescence, but three mutant colonies showed strong fluorescence after one day of growth. Sequencing revealed that the three mutant plasmids were distinct, but that all of them contained an N42H codon change. Therefore a variant was generated that had only the N42H substitution. The N42H variant was purified in parallel with DsRed1, and the two proteins were analyzed by spectrofluorometry. The spectra of purified DsRed1 changed over a period of days as the protein matured. By contrast, the spectra of the purified N42H variant remained stable over time consistent with rapid maturation. In addition to accelerating maturation, the N42H substitution altered the spectral properties of the mature protein. Mature DsRed1 was assumed to be an equilibrium mixture of red fluorescent molecules and some green fluorescent molecules that were spectrally similar to GFP. The GFP-like species had a blue excitation peak at approximately 480 nm and a green emission peak at approximately 500 nm, but DsRed was tetramer, so excitation of the green molecules often resulted in the fluorescence resonance energy transfer (FRET) with neighboring red molecules to produce and emission. The FRET effect, together with the relatively low percentage of green molecules in mature DsRed1, yielded a very small peak of green emission relative to the red emission. In the N42H variant, the peaks of blue excitation and green emission were dramatically enhanced, indicating that the equilibrium had shifted so that a target percentage of the mature molecules contained the green chromophore. Because the N42H substitution considerably increased the size of the side chain, a more conservative N42Q substitution was also tried. This mutation required two base changes and probably would not have been present in the original mutant collection. The N42Q variant retained the rapid maturation property of the N42H variant, but showed much less blue excitation and green emission. The N42Q variant was therefore chosen as the starting point for further study. Additional mutagenesis yielded DsRed variants that showed even faster maturation and lower green emission than the original N42Q variant. After six rounds of mutagenesis, three optimized variants were selected and termed DsRed T1, DsRed T3 and DsRed T4. The spectral properties of DsRed T4 were virtually identical to those of DsRed T1 and very similar to those of the wild-type DsRed1. Compared with DsRed T1 and DsRed T4, DsRed T3 was somewhat brighter but has a significantly higher peak of blue excitation and a marginally higher peak of green emission. The optimized DsRed variants were examined both in vivo and in vitro. As judged by colony fluorescence, colony size, and plasmid stability, these variants were less toxic to *E. coli* than DsRed1, and they developed fluorescence more efficiently at growth temperatures of 37 degreesC and higher. Like wild-type DsRed, the optimized variants appeared to be tetrameric, they exhibited FRET between the green and red molecules. The end result was pair of optimized variants termed DsRed T3 and DsRed T4. DsRed T4, DsRed T3 matured rapidly, and the purified protein was nearly as bright as mature wild-type DsRed. Making this variant well suited to single-color imaging of red fluorescence. DsRed T3 had a higher peak of blue excitation and a slightly higher peak of green emission than wild-type DsRed. DsRed T4 had fluorescence spectra very similar to those of wild-type DsRed and yielded negligible contamination of the GFP signal.

Although purified DsRed T4 was only about half as bright as DsRed T3, this effect was partially offset in vivo because DsRed T4 matures nearly twice as fast as DsRed T3. Thus, DsRed T4 was probably the best variant for most applications. (65 pages)

ACCESSION NUMBER: 2003-20948 BIOTECHDS

TITLE: Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins; involving vector-mediated gene transfer and expression in Escherichia coli

AUTHOR: BEVIS B; GLICK B

PATENT ASSIGNEE: UNIV CHICAGO

PATENT INFO: WO 2003054158 3 Jul 2003

APPLICATION INFO: WO 2002-US40539 18 Dec 2002

PRIORITY INFO: US 2001-341723 19 Dec 2001; US 2001-341723 19 Dec 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-569236 [53]

L4 ANSWER 4 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
TI New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines;

involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical and cosmetic industry

AN 2003-15503 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid encoding a polypeptide product comprising a first and a second chromo/fluorescent domain, optionally joined by a linking domain, is new. The first and second chromo/fluorescent domains associate with each other under intracellular conditions so that the encoded polypeptide assumes a tertiary structure.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a construct comprising a vector and the above nucleic acid; (2) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the nucleic acid cited above; (3) a cell, or its progeny, comprising an expression cassette cited above as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the cassette into the host cell; (4) producing the above polypeptide, comprising growing the cell cited above to express the polypeptide product; (5) a protein, or its fragment, encoded by the nucleic acid cited above; (6) an antibody binding specifically to the protein cited above; (7) a transgenic organism or transgenic cell or cell progeny, comprising a transgene that is the nucleic acid cited above; (8) an application that employs a chromo- or fluorescent protein or a nucleic acid encoding the chromo- or fluorescent protein, the improvement comprising employing the above protein or nucleic acid; and (9) a kit comprising the nucleic acid cited above.

BIOTECHNOLOGY - Preferred Nucleic Acid: The first and second chromo/fluorescent domains are oligomeric producing domains. The chromo/fluorescent domains are chromo- or fluorescent proteins from a Cnidarian species or mutants of chromo- or fluorescent proteins from a Cnidarian species. The Cnidarian species is a non-bioluminescent Cnidarian species, particularly an Anthozoan species. The nucleic acid encodes a fusion protein of the first and second chromo/fluorescent domains fused to a non-chromo/fluorescent protein domain.

USE - The nucleic acid and the protein are useful in producing labeled fusion proteins that have a precise and predictable signal to fusion partner ratio. The nucleic acid may also be used in generating transgenic, non-human plants or animals or site-specific gene modifications in cell lines. The chromoproteins may be used as coloring agents, as a food composition, in pharmaceuticals or cosmetics, as labels in analyte detection assays or as selectable markers in recombinant DNA applications. (34 pages)

ACCESSION NUMBER: 2003-15503 BIOTECHDS

TITLE: New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines; involving vector-mediated gene transfer and expression in host cell for use in color, food-additive, pharmaceutical and cosmetic industry

AUTHOR: LUKYANOV S A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2003031590 17 Apr 2003

APPLICATION INFO: WO 2002-US32560 10 Oct 2002

PRIORITY INFO: US 2002-383336 22 May 2002; US 2001-976673 12 Oct 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-381709 [36]

L4 ANSWER 5 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

AN 2003-09318 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment (II) of (I); (2) a construct (III) comprising a vector and (I); (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host; (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell; (5) a protein (VI) or its fragment encoded by (I); (6) an antibody (VII) binding specifically to (VI); (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I); (8) a transgenic organism (IX) comprising a transgene that comprises (I); (9) production of (VI); (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent; (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

WIDER DISCLOSURE - Also disclosed are: (1) a composition comprising (I), (V), (VI), (VII) or recombinant vectors; (2) homologs of (I) or (VI); (3) nucleic acids that encode the proteins encoded by (I), but differ in sequence from (I) due to the degeneracy of the genetic code; (4) nucleic acids that hybridize to (I) under stringent conditions; (5)

nucleic acids encoding variants e.g., mutants of (I); and (6) nucleic acids that encode fusion proteins of (I) or its fragments.

BIOTECHNOLOGY - Preparation: (VI) is produced by growing (V), where (VI) is expressed, and isolating (VI) that is substantially free of other proteins (claimed). Preferred Nucleic Acid: In (I), the kindling stimulus is light of a kindling wavelength, intensity and duration effective to kindle the kindling fluorescent protein. The kindling wavelength of the kindling stimulus ranges from about 200-1500 nm, the kindling intensity of the kindling stimulus ranges from 0.01-106 W/cm², and the kindling duration of the kindling stimulus ranges from 1 millisecond to about 60 minutes. The kindling fluorescent protein does not have a sequence that is identical to a sequence comprising 232 or 215 amino acids fully defined in the specification. The kindling fluorescent protein is a mutant of a wild type kindling fluorescent protein. The kindling fluorescent protein is a wild type protein or its mutant from a non-bioluminescent Cnidarian species, preferably an Anthozoan species. The second state is transient. The second fluorescent state is permanent. (I) is an isolated nucleic acid. Preferred Method: In (M), the kindling fluorescent protein is present inside an organism or cell.

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed). (I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

EXAMPLE - Generation of and initial characterization of kindling fluorescent proteins was as follows. Routine target i.e. site specific, and random mutagenesis of wild type asFP595 and *Heteractis crispa* chromoproteins was carried out. The asFP595 (asCP) had a sequence comprising 232 amino acids fully defined in the specification and was encoded by a nucleotide coding sequence comprising 767 base pairs fully defined in the specification. The *H.crispa* chromoprotein had a sequence comprising 215 amino acids fully defined in the specification was encoded by a nucleotide coding sequence comprising 760 base pairs fully defined in the specification. Mutagenesis was performed by the overlap extension method. Briefly, two overlapping fragments of each FP coding region were amplified. Polymerase chain reaction (PCR) was carried out using Advantage 2 polymerase mix in 1xmanufacturer's buffer supplemented with 100 μM of each dNTP, 0.2 μM of each primer, and 1 ng of plasmid DNA in 25 μl (final volume). To remove plasmids encoding wild type proteins, the 5' and 3'-fragments were excised from 2% low-melting agarose gel in 1xTAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. For each particular mutant, appropriate 5'- and 3'-fragments were combined to obtain full-length cDNA as follows. Equal volumes of 5'-fragment solution, 3'-fragment solution and 3xPCR mixture containing Advantage 2 polymerase mix, buffer and dNTPs were mixed together and subjected to 2-3 cycles of 95°C for 20 s, 65°C for 30 min, 72°C for 30 s. Then, the reaction was diluted 10 fold

and 1 μl of the diluted sample was used as a template for PCR with forward and reverse cloning primers. As a result, ready-for-cloning fragments containing full-length coding regions with target substitution(s) were generated. Mutant PC products were digested with endonucleases, for which the cloning primers contained sites, and then cloned into pQE30, digested with endonucleases generating complementary overhangs. Each of the recombinant proteins generated by both cloning-expression systems contained a 6xHis tag on the N- or C-terminus. Selected Escherichia coli clones were grown at 37°C in 50 ml to an optical density of (OD) 0.6. At that point, the expression of recombinant FP was induced with 0.2 mM isopropyl-beta-D-thiogalactoside (IPTG). The cultures were then incubated overnight. The following day, cells were harvested by centrifugation, resuspended in buffer and disrupted by sonication. Fluorescent proteins were purified from the soluble fraction. Proteins were at least 95% pure according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (96 pages)

ACCESSION NUMBER: 2003-09318 BIOTECHDS

TITLE: Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms; vector-mediated recombinant protein gene transfer and expression in host cell for use in fluorescence resonance energy transfer, luminescence resonance energy transfer, high throughput screening and cell sorting

AUTHOR: LUKYANOV S A; CHUDAKOV D; LUKYANOV K

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2002096924 5 Dec 2002

APPLICATION INFO: WO 2002-US16379 24 May 2002

PRIORITY INFO: US 2001-329176 11 Oct 2001; US 2001-293752 25 May 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-156788 [15]

L4 ANSWER 6 OF 16 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN

TI New nucleic acid encoding a non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant for analyte detection assays or fluorescence activated cell sorting applications; vector-mediated reporter gene transfer and expression in host cell or transgenic animal for biosensor construction

AN 2003-03300 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A nucleic acid which encodes a non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a fragment of the novel nucleic acid; (2) a construct comprising a vector and the novel nucleic acid; (3) an expression cassette comprising transcriptional initiation and termination regions functional in an expression host, and the novel nucleic acid; (4) a cell, or its progeny, comprising the expression cassette of (3) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell; (5) producing a chromo- and/or fluorescent protein, comprising growing a cell of (4), where the protein is expressed, and isolating the protein free of other proteins; (6) a protein, or its fragment, encoded by the novel nucleic acid; (7) an antibody that binds specifically to the protein of (6); (8) a transgenic cell, or its progeny, or a transgenic organism comprising a transgene that is the above nucleic acid; (9) in an application that employs a chromo- or fluorescent protein, the improvement comprising employing the

protein of (6) or the novel nucleic acid; (10) a kit comprising the novel nucleic acid; and (11) producing the novel nucleic acid, comprising modulating at least one N-terminal residue codon of an aggregating Cnidarian chromo- and/or fluorescent protein encoding sequence to produce the nucleic acid.

BIOTECHNOLOGY - Preferred Nucleic Acid: The nucleic acid has a sequence of residues that is substantially the same as or identical to at least 10 residues in length of a 678, 690, 707, 654 or 705 base pair sequence, given in the specification. The Cnidarian chromo- or fluorescent protein is from a non-bioluminescent Cnidarian species, specifically an Anthozoan species. Preferred Method: In producing the novel nucleic acid, the residue is a basic residue, specifically, Lys or Arg. The modulation is a substitution of the basic residue for a neutral residue.

USE - The nucleic acid is useful in encoding Cnidarian chromo- or fluorescent protein useful in analyte detection assays, as coloring agents, as markers in recombinant DNA applications, as a sunscreen or filter, in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in screening assays, as second messenger detectors, in fluorescence activated cell sorting applications, in protease cleavage assays, or as a fluorescent timer.(80 pages)

ACCESSION NUMBER: 2003-03300 BIOTECHDS

TITLE: New nucleic acid encoding a non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant for analyte detection assays or fluorescence activated cell sorting applications; vector-mediated reporter gene transfer and expression in host cell or transgenic animal for biosensor construction

AUTHOR: LUKYANOV S; LUKYANOV K; YANUSHEVICH Y; SAVISTKY A; FRADKOV A

PATENT ASSIGNEE: CLONTECH LAB INC

PATENT INFO: WO 2002068459 6 Sep 2002

APPLICATION INFO: WO 2002-US5749 20 Feb 2002

PRIORITY INFO: US 2001-6922 4 Dec 2001; US 2001-270983 21 Feb 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2002-691654 [74]

L4 ANSWER 7 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN
TI Rapidly maturing fluorescent proteins and methods for using the same
AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. red fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:588435 HCAPLUS

DOCUMENT NUMBER: 143:112117
 TITLE: Rapidly maturing fluorescent proteins and methods for using the same
 INVENTOR(S): Bevis, Brooke; Glick, Benjamin
 PATENT ASSIGNEE(S): The University of Chicago, USA
 SOURCE: U.S. Pat. Appl. Publ., 28 pp., Cont.-in-part of Appl. No. PCT/US02/40539.
 CODEN: USXXCO
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|-------------|
| US 2005149994 | A1 | 20050707 | US 2004-844064 | 20040511 |
| WO 2003054158 | A2 | 20030703 | WO 2002-US40539 | 20021218 |
| WO 2003054158 | A3 | 20031204 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| PRIORITY APPLN. INFO.: | | | US 2001-341723P | P 20011219 |
| | | | WO 2002-US40539 | A2 20021218 |

L4 ANSWER 8 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI Novel Cnidarian and Anthozoan chromo/fluoroproteins and cDNA encoding them
 AB DNA encoding novel chromo/fluoroproteins as well as the encoded proteins are provided. The proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian , e.g., Anthozoan , species or are obtained from Anthozoan non- Pennatulacean (sea pen) species. Specific proteins of interest include the following specific proteins: Heteractis crispa hcriGFP; Dendronephthya dendGFP; Zoanthus zoanRFP; Scolymia cubensis scubGFP1 and scubGFP2; Ricordea florida rfloRFP, rfloGFP2, and rfloGFP; Montastraea cavernosa mcavRFP, mcavGFP, and mcavGFP2; Condylactis gigantea cgigGFP; Agaricia fragilis afraGFP; and Montastraea annularis mannFP. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2005:122690 HCAPLUS
 DOCUMENT NUMBER: 142:193054
 TITLE: Novel Cnidarian and Anthozoan chromo/fluoroproteins and cDNA encoding them
 INVENTOR(S): Labas, Yuliia Aleksandrovich; Gurskaya, Nadezda Georgievna; Yanushevich, Yuriy; Fradkov, Arcady Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey; Matz, Mikhail Vladimirovich
 PATENT ASSIGNEE(S): Russia

SOURCE: U.S. Pat. Appl. Publ., 63 pp., Cont.-in-part of Appl.
 No. PCT/02US/36499.
 CODEN: USXXCO

DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|-------------|
| US 2005032085 | A1 | 20050210 | US 2004-757356 | 20040113 |
| WO 2003042401 | A2 | 20030522 | WO 2002-US36499 | 20021112 |
| WO 2003042401 | A3 | 20031120 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| PRIORITY APPLN. INFO.: | | | US 2001-332980P | P 20011113 |
| | | | WO 2002-US36499 | A2 20021112 |

L4 ANSWER 9 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
 AB Nucleic acid compns. encoding rapidly maturing fluorescent proteins, as well as non-aggregating versions thereof (and mutants thereof) as well as the proteins encoding the same, are provided. The proteins of interest are proteins that are fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that, in certain embodiments, they are mutants of wild type proteins that are obtained either from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from Anthozoan non-Pennatulacean (sea pen) species. In certain embodiments, the subject proteins are mutants of wild type Discosoma sp. 'red' fluorescent protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided. Claimed sequences were not present at the time of publication.

ACCESSION NUMBER: 2003:511470 HCAPLUS
 DOCUMENT NUMBER: 139:65739
 TITLE: Engineering rapidly maturing variants of the Discosoma red fluorescent protein (DsRed) and their use as bioluminescent probes
 INVENTOR(S): Bevis, Brooke; Glick, Benjamin
 PATENT ASSIGNEE(S): The University of Chicago, USA
 SOURCE: PCT Int. Appl., 65 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| WO 2003054158 | A2 | 20030703 | WO 2002-US40539 | 20021218 |
| WO 2003054158 | A3 | 20031204 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ,
UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES,
FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ,
CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2467383 | AA | 20030703 | CA 2002-2467383 | 20021218 |
| AU 2002357322 | A1 | 20030709 | AU 2002-357322 | 20021218 |
| EP 1456223 | A2 | 20040915 | EP 2002-805620 | 20021218 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK | | | | |
| JP 2006501804 | T2 | 20060119 | JP 2003-554863 | 20021218 |
| US 2005149994 | A1 | 20050707 | US 2004-844064 | 20040511 |
| PRIORITY APPLN. INFO.: US 2001-341723P P 20011219
WO 2002-US40539 W 20021218 | | | | |

L4 ANSWER 10 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN
 TI cDNAs encoding chromo/fluoroproteins from non-bioluminescent
 Cnidarian species or non-Pennatulacean (sea pen) species and their
 use
 AB Nucleic acid compns. encoding novel
 chromo/fluoroproteins and mutants thereof, as well as the proteins encoded
 the same, are provided. The proteins of interest are proteins that are
 colored and/or fluorescent, where this feature arises from the interaction
 of two or more residues of the protein. The subject proteins are further
 characterized in that they are either obtained from non-bioluminescent
 Cnidarian, e.g., Anthozoan, species or are obtained from
 Anthozoan non-Pennatulacean (sea pen) species. More specifically,
 they include GFP of *Heteractis crispa*, *Dendronephthya* sp., *Scolymia*
cubensis, *Ricordea florida*, *Montastraea cavernosa*, *Condylactis gigantea*,
Agaricia fragilis, sequence homolog of *Monistraea annularis* and RFP of
Zoanthus sp., *Ricordea florida*, and *Montastraea cavernosa*. Also of
 interest are proteins that are substantially similar to, or mutants of,
 the above specific proteins. Also provided are fragments of the nucleic
 acids and the peptides encoded thereby, as well as antibodies to the
 subject proteins and transgenic cells and organisms. The subject protein
 and nucleic acid compns. find use in a variety of
 different applications. Finally, kits for use in such applications, e.g.,
 that include the subject nucleic acid compns., are
 provided.

ACCESSION NUMBER: 2003:397030 HCAPLUS
 DOCUMENT NUMBER: 138:397335
 TITLE: cDNAs encoding chromo/fluoroproteins from
 non-bioluminescent Cnidarian species or
 non-Pennatulacean (sea pen) species and their use
 INVENTOR(S): Labas, Yulii Aleksandrovich; Gurskaya, Nadezda
 Georgievna; Yanushevich, Yuriy; Fradkov, Arcady
 Fedorovich; Lukyanov, Konstantin; Lukyanov, Sergey;
 Matz, Mikhail Vladimirovich
 PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA
 SOURCE: PCT Int. Appl., 88 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|------------|
| WO 2003042401 | A2 | 20030522 | WO 2002-US36499 | 20021112 |
| WO 2003042401 | A3 | 20031120 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2454031 | AA | 20030522 | CA 2002-2454031 | 20021112 |
| EP 1444245 | A2 | 20040811 | EP 2002-797104 | 20021112 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK | | | | |
| JP 2005509420 | T2 | 20050414 | JP 2003-544215 | 20021112 |
| US 2005032085 | A1 | 20050210 | US 2004-757356 | 20040113 |
| PRIORITY APPLN. INFO.: | | | US 2001-332980P | P 20011113 |
| | | | WO 2002-US36499 | W 20021112 |

L4 ANSWER 11 OF 16 HCAPLUS COPYRIGHT 2006 ACS on STN

TI cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same

AB Nucleic acid compns. encoding novel chromo/fluoroproteins and mutants thereof, as well as the proteins encoded by the same, are provided. The subject proteins of interest are proteins that are colored and/or fluorescent, where this feature arises from the interaction of two or more residues of the protein. The subject proteins are further characterized in that they are either obtained from non-bioluminescent Cnidarian, e.g., Anthozoan, species or are obtained from non-Pennatulacean (sea pen) species. Specific proteins of interest include proteins obtained from the following specific Anthozoan species: *Anemonia majano* (NFP-1), *Clavularia* sp. (NFP-2), *Zoanthus* sp. (NFP-3 & NFP-4), *Discosoma striata* (NFP-5), *Discosoma* sp. "red" (NFP-6), *Anemonia sulcata* (NFP-7), *Discosoma* sp "green" (NFP-8), and *Discosoma* sp. "magenta" (NFP-9). Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compns. find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compns., are provided.

ACCESSION NUMBER: 2002:978391 HCAPLUS
 DOCUMENT NUMBER: 138:50935
 TITLE: cDNA and protein sequences of novel chromo/fluoroproteins from non-bioluminescent Cnidarian species or are obtained from non-Pennatulacean (sea pen) species and methods for using the same
 INVENTOR(S): Lukyanov, Sergey A.; Fradkov, Arcady F.; Labas, Yulii A.; Matz, Mikhail V.; Terskikh, Alexey
 PATENT ASSIGNEE(S): Russia
 SOURCE: U.S. Pat. Appl. Publ., 48 pp., Cont.-in-part of Appl. No. PCT/US00/28477.
 CODEN: USXXCO

DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 17
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|-----------------|-----------------|----------|
| US 2002197676 | A1 | 20021226 | US 2001-6922 | 20011204 |
| WO 2000034526 | A1 | 20000615 | WO 1999-US29405 | 19991210 |
| W: JP | | | | |
| RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| WO 2001027150 | A2 | 20010419 | WO 2000-US28477 | 20001013 |
| WO 2001027150 | A3 | 20011206 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2434737 | AA | 20020906 | CA 2002-2434737 | 20020220 |
| WO 2002068459 | A2 | 20020906 | WO 2002-US5749 | 20020220 |
| WO 2002068459 | A3 | 20031127 | | |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW | | | | |
| RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2002254031 | A1 | 20020912 | AU 2002-254031 | 20020220 |
| US 2003022287 | A1 | 20030130 | US 2002-81864 | 20020220 |
| US 6969597 | B2 | 20051129 | | |
| EP 1385967 | A2 | 20040204 | EP 2002-723238 | 20020220 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR | | | | |
| JP 2004536571 | T2 | 20041209 | JP 2002-567969 | 20020220 |
| US 2003092884 | A1 | 20030515 | US 2002-155809 | 20020524 |
| US 2006035330 | A1 | 20060216 | US 2005-187622 | 20050721 |
| AU 2006200881 | A1 | 20060330 | AU 2006-200881 | 20060301 |
| PRIORITY APPLN. INFO.: | | | | |
| | | US 1999-418529 | A2 | 19991014 |
| | | US 1999-418917 | B2 | 19991015 |
| | | US 1999-418922 | B2 | 19991015 |
| | | US 1999-444338 | B2 | 19991119 |
| | | US 1999-444341 | B2 | 19991119 |
| | | US 1999-457556 | B2 | 19991209 |
| | | US 1999-457898 | B2 | 19991209 |
| | | US 1999-458144 | B2 | 19991209 |
| | | US 1999-458477 | B2 | 19991209 |
| | | WO 1999-US29405 | W | 19991210 |
| | | US 2000-211607P | P | 20000614 |
| | | US 2000-211609P | P | 20000614 |
| | | US 2000-211626P | P | 20000614 |
| | | US 2000-211627P | P | 20000614 |
| | | US 2000-211687P | P | 20000614 |
| | | US 2000-211766P | P | 20000614 |
| | | US 2000-211880P | P | 20000614 |

| | | | |
|----|--------------|----|----------|
| US | 2000-211888P | P | 20000614 |
| US | 2000-212070P | P | 20000614 |
| WO | 2000-US28477 | A2 | 20001013 |
| US | 1998-210330 | A | 19981211 |
| AU | 2001-10867 | A3 | 20001013 |
| US | 2001-270983P | P | 20010221 |
| US | 2001-293752P | P | 20010525 |
| US | 2001-329176P | P | 20011011 |
| US | 2001-976673 | A | 20011012 |
| US | 2001-6922 | A | 20011204 |
| US | 2002-81864 | A1 | 20020220 |
| WO | 2002-US5749 | W | 20020220 |

L4 ANSWER 12 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
 TI Novel nucleic acid encoding interconverted mutant of chromo- or fluorescent protein which are useful as biosensors, coloring agents.

AN 2003-607998 [57] WPIDS

AB WO2003057833 A UPAB: 20030906

NOVELTY - Nucleic acid encoding an interconverted mutant (I) of a chromo- or fluorescent protein, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) a fragment of a nucleic acid encoding (I);

(2) a construct comprising a vector and a nucleic acid encoding (I);

(3) an expression cassette (II) comprises, a transcriptional initiation region that is functional in an expression host, a nucleic acid encoding (I) and a transcriptional termination region functional in the expression host;

(4) a cell or its progeny comprising (II), as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of (II) into the host cell;

(5) producing a chromo and/or fluorescent protein, comprises, growing the cell where protein is expressed and isolating the protein substantially free of other proteins;

(6) a protein (III) or its fragment encoded by the nucleic acid encoding (I) and an antibody binding specifically to the (III);

(7) transgenic cell or its progeny comprises a transgene which is a nucleic acid encoding (I);

(8) a kit comprising a nucleic acid encoding (I);

(9) preparation (M1) of nucleic acid encoding (I); and

(10) a nucleic acid produced by (M1).

USE - Nucleic acid encoding (I) is useful in any application that employs a chromo- or fluorescent protein. (III) is useful in any application that employs a chromo- or fluorescent protein (claimed). Nucleic acid encoding (I) is useful in the generation of transgenic, non-human plants or animals or site specific gene modification in cell lines. Chromoprotein encoded by the nucleic acid is useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, etc. The chromoprotein is also useful as labels in biological analyte detection assays and as selectable markers in recombinant DNA applications (e.g. the production of transgenic cells and organisms) and is also useful as sunscreens, selective filters, etc. The fluorescent protein encoded by the nucleic acid, is useful in fluorescence resonance energy transfer (FRET) applications and also useful as biosensors in prokaryotic and eukaryotic cells e.g. as Ca²⁺ ion indicator and as marker of whole cells to detect changes in multicellular reorganization and migration. The fluorescent proteins are also useful as second messenger detector, e.g. by fusing the subject proteins to specific

domains (Protein Kinase C gamma calcium binding domain) and as in vivo marker in animals (e.g. transgenic animals). The fluorescent proteins are also useful in fluorescence activated cell sorting application, in protease cleavage assays and in assays to determine the phospholipid composition in biological membranes. The fluorescent protein is a fluorescent timer, where the switch of one fluorescent color to another (e.g. green to red) concomitant with the aging of fluorescent protein, is used to determine the activation or deactivation of gene expression.

DESCRIPTION OF DRAWING(S) - The figure shows the normalized spectra for selected mutants of asCP and DsRed.

Dwg.3/3

ACCESSION NUMBER: 2003-607998 [57] WPIDS
 DOC. NO. CPI: C2003-165725
 TITLE: Novel nucleic acid encoding interconverted mutant of chromo-or fluorescent protein which are useful as biosensors, coloring agents.
 DERWENT CLASS: B04 D16
 INVENTOR(S): BULINA, M E; CHUDAKOV, D; LUKYANOV, K A
 PATENT ASSIGNEE(S): (CLON-N) CLONTECH LAB INC; (BULI-I) BULINA M E; (CHUD-I) CHUDAKOV D; (LUKY-I) LUKYANOV K A
 COUNTRY COUNT: 103
 PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---------------|---|--------------------|------|----|----|
| WO 2003057833 | A2 | 20030717 (200357)* | EN | 56 | |
| RW: | AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW | | | | |
| W: | AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW | | | | |
| AU 2002367391 | A1 | 20030724 (200421) | | | |
| US 2004248180 | A1 | 20041209 (200481) | | | |
| EP 1504017 | A2 | 20050209 (200512) | EN | | |
| R: | AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR | | | | |
| JP 2005514032 | W | 20050519 (200538) | | 38 | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|--------------------------|--|----------------------------------|
| WO 2003057833 | A2 | WO 2002-US41418 | 20021223 |
| AU 2002367391 | A1 | AU 2002-367391 | 20021223 |
| US 2004248180 | A1 Provisional
CIP of | US 2001-343128P
WO 2002-US41418
US 2004-845484 | 20011226
20021223
20040512 |
| EP 1504017 | A2 | EP 2002-806227
WO 2002-US41418 | 20021223
20021223 |
| JP 2005514032 | W | WO 2002-US41418
JP 2003-558135 | 20021223
20021223 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---------------|-------------|---------------|
| AU 2002367391 | A1 Based on | WO 2003057833 |
| EP 1504017 | A2 Based on | WO 2003057833 |
| JP 2005514032 | W Based on | WO 2003057833 |

PRIORITY APPLN. INFO: US 2001-343128P 20011226; US

L4 ANSWER 13 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins.
AN 2003-569236 [53] WPIDS
AB WO2003054158 A UPAB: 20030820
NOVELTY - A nucleic acid (I) that encodes a rapidly maturing chromo or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising, a transcriptional initiation region functional in an expression host, (I), or (II), and a transcriptional termination region functional in the expression host;
- (4) a cell (V), or its progeny, comprising (IV) as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the expression cassette into the host cell;
- (5) a protein (VI) or its fragment encoded by (I);
- (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell or its progeny, or a transgenic organism comprising a transgene that is (I) or (II); and
- (8) a kit comprising (I) or (II).

USE - (I) is useful in applications involving nucleic acid encoding a chromo- or fluorescent protein. (V) is useful for producing a chromo and/or fluorescent protein which involves growing the cell, whereby the protein is expressed, and isolating the protein substantially free of other proteins. (VI) is useful in applications involving chromo- or fluorescent protein (claimed).

(I) is useful as PCR primers, hybridization probes, etc. The expression cassettes are useful for synthesizing (VI). The chromoproteins are useful as coloring agents which are capable of imparting color or pigment to a particular composition of matter e.g. food compositions, pharmaceuticals, cosmetics, living organisms, e.g., animals and plants. The chromoproteins may also find use as labels in analyte detection assays, e.g. assays for biological analytes of interest and as selectable markers in recombinant DNA applications, e.g. the production of transgenic cells and organisms. The fluorescent proteins find use in a variety of different applications, e.g. in fluorescence resonance energy transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups by using microscopic imaging and electronic analysis, as second messenger detectors, and in fluorescence activated cell sorting applications and as in vivo marker in animals. The fluorescent proteins also find use in protease cleavage assays. The proteins can also be used in assays to determine the phospholipid composition in biological membranes and as a fluorescent timer.

Dwg. 0/4

ACCESSION NUMBER: 2003-569236 [53] WPIDS
DOC. NO. CPI: C2003-153632
TITLE: Novel nucleic acid encoding a rapidly maturing chromo- or fluorescent mutant of a Cnidarian chromo- or fluorescent protein or its mutant, useful for applications involving chromo- or fluorescent proteins.
DERWENT CLASS: B04 D16
INVENTOR(S): BEVIS, B; GLICK, B
PATENT ASSIGNEE(S): (UYCH-N) UNIV CHICAGO
COUNTRY COUNT: 103
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|--|------|----------|-----------|----|----|
| WO 2003054158 | A2 | 20030703 | (200353)* | EN | 65 |
| RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW | | | | | |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW | | | | | |
| AU 2002357322 | A1 | 20030709 | (200428) | | |
| EP 1456223 | A2 | 20040915 | (200460) | EN | |
| R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC
MK NL PT RO SE SI SK TR | | | | | |
| US 2005149994 | A1 | 20050707 | (200547) | | |
| JP 2006501804 | W | 20060119 | (200606) | | 43 |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|--------------------------|-----------------|----------|
| WO 2003054158 | A2 | WO 2002-US40539 | 20021218 |
| AU 2002357322 | A1 | AU 2002-357322 | 20021218 |
| EP 1456223 | A2 | EP 2002-805620 | 20021218 |
| | | WO 2002-US40539 | 20021218 |
| US 2005149994 | A1 Provisional
CIP of | US 2001-341723P | 20011219 |
| | | WO 2002-US40539 | 20021218 |
| | | US 2004-844064 | 20040511 |
| JP 2006501804 | W | WO 2002-US40539 | 20021218 |
| | | JP 2003-554863 | 20021218 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---------------|-------------|---------------|
| AU 2002357322 | A1 Based on | WO 2003054158 |
| EP 1456223 | A2 Based on | WO 2003054158 |
| JP 2006501804 | W Based on | WO 2003054158 |

PRIORITY APPLN. INFO: US 2001-341723P 20011219; US
2004-844064 20040511

L4 ANSWER 14 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI New nucleic acid encoding polypeptide products having
at least two linked chromo/fluorescent domains, useful for generating
transgenic plants or animals or site-specific gene modifications in cell
lines.

AN 2003-381709 [36] WPIDS

CR 2002-444170 [47]

AB WO2003031590 A UPAB: 20050411

NOVELTY - A nucleic acid encoding a polypeptide
product comprising a first and a second chromo/fluorescent domain,
optionally joined by a linking domain, is new. The first and second
chromo/fluorescent domains associate with each other under intracellular
conditions so that the encoded polypeptide assumes a tertiary structure.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the
following:

(1) a construct comprising a vector and the above nucleic
acid;

(2) an expression cassette comprising transcriptional initiation and
termination regions functional in an expression host, and the
nucleic acid cited above;

(3) a cell, or its progeny, comprising an expression cassette cited above as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of the cassette into the host cell;

(4) producing the above polypeptide, comprising growing the cell cited above to express the polypeptide product;

(5) a protein, or its fragment, encoded by the nucleic acid cited above;

(6) an antibody binding specifically to the protein cited above;

(7) a transgenic organism or transgenic cell or cell progeny, comprising a transgene that is the nucleic acid cited above;

(8) an application that employs a chromo- or fluorescent protein or a nucleic acid encoding the chromo- or fluorescent protein, the improvement comprising employing the above protein or nucleic acid; and

(9) a kit comprising the nucleic acid cited above.

USE - The nucleic acid and the protein are useful in producing labeled fusion proteins that have a precise and predictable signal to fusion partner ratio. The nucleic acid may also be used in generating transgenic, non-human plants or animals or site-specific gene modifications in cell lines. The chromoproteins may be used as coloring agents, as a food composition, in pharmaceuticals or cosmetics, as labels in analyte detection assays or as selectable markers in recombinant DNA applications.

Dwg.0/7

ACCESSION NUMBER: 2003-381709 [36] WPIDS
CROSS REFERENCE: 2002-444170 [47]
DOC. NO. CPI: C2003-101460
TITLE: New nucleic acid encoding polypeptide products having at least two linked chromo/fluorescent domains, useful for generating transgenic plants or animals or site-specific gene modifications in cell lines.
DERWENT CLASS: B04 D13 D16
INVENTOR(S): LUKYANOV, S A
PATENT ASSIGNEE(S): (CLON-N) CLONTECH LAB INC
COUNTRY COUNT: 102
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---------------|---|--------------------|------|-----|----|
| WO 2003031590 | A2 | 20030417 (200336)* | EN | 34 | |
| RW: | AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW | | | | |
| W: | AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW | | | | |
| EP 1434483 | A2 | 20040707 (200444) | EN | | |
| R: | AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR | | | | |
| AU 2002362771 | A1 | 20030422 (200460) | | | |
| US 2004216180 | A1 | 20041028 (200471) | | | |
| JP 2005507657 | W | 20050324 (200523) | | 105 | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|------|-----------------|----------|
| WO 2003031590 | A2 | WO 2002-US32560 | 20021010 |

| | | | |
|---------------|---|---|--|
| EP 1434483 | A2 | EP 2002-801039 | 20021010 |
| AU 2002362771 | A1 | WO 2002-US32560 | 20021010 |
| US 2004216180 | A1 CIP of
Provisional
Provisional
CIP of | AU 2002-362771
US 2001-976673
US 2002-356225P
US 2002-383336P
WO 2002-US32560
US 2004-806930 | 20021010
20011012
20020211
20020522
20021010
20040322 |
| JP 2005507657 | W | WO 2002-US32560
JP 2003-534561 | 20021010
20021010 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|---------------|-------------|---------------|
| EP 1434483 | A2 Based on | WO 2003031590 |
| AU 2002362771 | A1 Based on | WO 2003031590 |
| JP 2005507657 | W Based on | WO 2003031590 |

PRIORITY APPLN. INFO: US 2002-383336P 20020522; US
 2001-976673 20011012; US
 2002-356225P 20020211; US
 2004-806930 20040322

L4 ANSWER 15 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
 TI Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms.
 AN 2003-156788 [15] WPIDS
 AB WO 200296924 A UPAB: 20030303
 NOVELTY - A nucleic acid (I) present in other than its natural environment, where (I) encodes a kindling fluorescent protein that goes from a first substantially non-fluorescent or non-fluorescent state to a second fluorescent state upon exposure to a kindling stimulus, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) a fragment (II) of (I);
- (2) a construct (III) comprising a vector and (I);
- (3) an expression cassette (IV) comprising a transcriptional initiation region functional in an expression host, (I), and a transcriptional termination region functional in the expression host;
- (4) a cell (V) or its progeny comprising (IV) as a part of an extrachromosomal element or integrated into the genome of a host cell;
- (5) a protein (VI) or its fragment encoded by (I);
- (6) an antibody (VII) binding specifically to (VI);
- (7) a transgenic cell (VIII) or its progeny comprising a transgene that comprises (I);
- (8) a transgenic organism (IX) comprising a transgene that comprises (I);
- (9) production of (VI);
- (10) producing (M) a fluorescent protein by subjecting (I) to a kindling stimulus to produce a kindled kindling fluorescent protein which is fluorescent;
- (11) a system (X) for producing a kindled fluorescent protein from (VI), comprises (I) or (VI), and a source of kindling stimulus; and
- (12) a kit (XI) comprising (I) and instructions for producing a fluorescent protein from (I).

USE - (VI) is useful for detecting an entity such as a protein, organelle or cell in a composition such as a cell or a multicellular composition (preferably a multicellular organism), by providing the entity as an entity labeled with (VI), kindling the kindling fluorescent protein

label with a kindling stimulus to produce a kindled kindling fluorescent protein label, and exciting the kindled kindling fluorescent protein label with light and detecting any fluorescence from it to detect the entity. The method monitors the movement of the entity (claimed).

(I) or (VI) is useful in labeling protocols, e.g., labeling proteins, organelles, cells and organisms, as biological labels or markers, in protein labeling or tagging applications. (II) is useful as primers for polymerase chain reaction, as hybridization screening probes and for the production of (VI). (VI) is useful as detectable labels, as labels in analyte detection assays, in fluorescence resonance energy transfer (FRET) applications, in bioluminescence resonance energy transfer (BRET) applications, as biosensors in prokaryotic and eukaryotic cells, in applications involving the automated screening of arrays of cells expressing fluorescent reporting groups, in high through-put screening assays, as second messenger detectors, and in fluorescent activated cell sorting assays.

Dwg.0/10

ACCESSION NUMBER: 2003-156788 [15] WPIDS
DOC. NO. CPI: C2003-040710
TITLE: Novel nucleic acid that is present in other than its natural environment and that encodes kindling fluorescent protein, is useful in labeling protocols, e.g. labeling proteins, organelles, cells and organisms.
DERWENT CLASS: B04 D16
INVENTOR(S): CHUDAKOV, D; LUKYANOV, K; LUKYANOV, S A
PATENT ASSIGNEE(S): (CHUD-I) CHUDAKOV D; (LUKY-I) LUKYANOV K; (LUKY-I) LUKYANOV S A; (CLON-N) CLONTECH LAB INC
COUNTRY COUNT: 101
PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
|---|------|--------------------|------|----|----|
| WO 2002096924 | A1 | 20021205 (200315)* | EN | 96 | |
| RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW | | | | | |
| W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW | | | | | |
| US 2003092884 | A1 | 20030515 (200335) | | | |
| EP 1390379 | A1 | 20040225 (200415) | EN | | |
| R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR | | | | | |
| AU 2002316164 | A1 | 20021209 (200452) | | | |

APPLICATION DETAILS:

| PATENT NO | KIND | APPLICATION | DATE |
|---------------|----------------|-----------------|----------|
| WO 2002096924 | A1 | WO 2002-US16379 | 20020524 |
| US 2003092884 | A1 Provisional | US 2001-293752P | 20010525 |
| | Provisional | US 2001-329176P | 20011011 |
| | | US 2002-155809 | 20020524 |
| EP 1390379 | A1 | EP 2002-746443 | 20020524 |
| | | WO 2002-US16379 | 20020524 |
| AU 2002316164 | A1 | AU 2002-316164 | 20020524 |

FILING DETAILS:

| PATENT NO | KIND | PATENT NO |
|-----------|------|-----------|
| | | |

EP 1390379 A1 Based on WO 2002096924
AU 2002316164 A1 Based on WO 2002096924

PRIORITY APPLN. INFO: US 2001-329176P 20011011; US
2001-293752P 20010525; US
2002-155809 20020524

L4 ANSWER 16 OF 16 WPIDS COPYRIGHT 2006 THE THOMSON CORP on STN
TI New nucleic acid encoding a non-aggregating chromo- or
fluorescent mutant of an aggregating Cnidarian chromo- or
fluorescent protein or mutant for analyte detection assays or fluorescence
activated cell sorting applications.

AN 2002-691654 [74] WPIDS

CR 2000-423373 [36]; 2000-423374 [36]; 2000-423375 [36]; 2000-423376 [36];
2000-423377 [36]; 2000-423378 [36]; 2000-423379 [36]; 2000-423380 [36];
2000-423381 [36]; 2000-423451 [36]; 2001-266409 [27]; 2002-154595 [20]

AB WO 200268459 A UPAB: 20060302

NOVELTY - A nucleic acid which encodes a
non-aggregating chromo- or fluorescent mutant of an aggregating
Cnidarian chromo- or fluorescent protein or mutant, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the
following:

- (1) a fragment of the novel nucleic acid;
- (2) a construct comprising a vector and the novel nucleic
acid;
- (3) an expression cassette comprising transcriptional initiation and
termination regions functional in an expression host, and the novel
nucleic acid;
- (4) a cell, or its progeny, comprising the expression cassette of (3)
as part of an extrachromosomal element or integrated into the genome of a
host cell as a result of introduction of the expression cassette into the
host cell;
- (5) producing a chromo- and/or fluorescent protein, comprising
growing a cell of (4), where the protein is expressed, and isolating the
protein free of other proteins;
- (6) a protein, or its fragment, encoded by the novel nucleic
acid;
- (7) an antibody that binds specifically to the protein of (6);
- (8) a transgenic cell, or its progeny, or a transgenic organism
comprising a transgene that is the above nucleic acid;
- (9) in an application that employs a chromo- or fluorescent protein,
the improvement comprising employing the protein of (6) or the novel
nucleic acid;
- (10) a kit comprising the novel nucleic acid; and
- (11) producing the novel nucleic acid, comprising
modulating at least one N-terminal residue codon of an aggregating
Cnidarian chromo- and/or fluorescent protein encoding sequence to
produce the nucleic acid.

USE - The nucleic acid is useful in encoding
Cnidarian chromo- or fluorescent protein useful in analyte
detection assays, as coloring agents, as markers in recombinant DNA
applications, as a sunscreen or filter, in fluorescence resonance energy
transfer (FRET) applications, as biosensors in prokaryotic and eukaryotic
cells, in screening assays, as second messenger detectors, in fluorescence
activated cell sorting applications, in protease cleavage assays, or as a
fluorescent timer.

Dwg. 0/16

ACCESSION NUMBER: 2002-691654 [74] WPIDS
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2000-423451 [36]; 2001-266409 [27]; 2002-154595 [20]

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TITLE: New nucleic acid encoding a non-aggregating chromo- or fluorescent mutant of an aggregating Cnidarian chromo- or fluorescent protein or mutant for analyte detection assays or fluorescence activated cell sorting applications.
 DERWENT CLASS: B04 D16 D21 D22
 INVENTOR(S): FRADKOV, A F; LABAS, Y A; LUKYANOV, S A; MATZ, M V;
 TERSKIKH, A; FRADKOV, A; LUKYANOV, K; LUKYANOV, S;
 SAVITSKY, A; YANUSHEVICH, Y; SAVISTKY, A
 PATENT ASSIGNEE(S): (FRAD-I) FRADKOV A F; (LABA-I) LABAS Y A; (LUKY-I)
 LUKYANOV S A; (MATZ-I) MATZ M V; (TERS-I) TERSKIKH A;
 (FRAD-I) FRADKOV A; (LUKY-I) LUKYANOV K; (LUKY-I)
 LUKYANOV S; (SAVI-I) SAVITSKY A; (YANU-I) YANUSHEVICH Y;
 (CLON-N) CLONTECH LAB INC
 COUNTRY COUNT: 101
 PATENT INFORMATION:

| PATENT NO | KIND | DATE | WEEK | LA | PG |
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| WO 2002068459 | A2 | 20020906 (200274)* | EN | 80 | |
| RW: | AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW | | | | |
| W: | AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW | | | | |
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| AU 2002254031 | A1 | 20020912 (200433) | | | |
| JP 2004536571 | W | 20041209 (200481) | | 208 | |
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| WO 2002068459 | A2 | WO 2002-US5749 | 20020220 |
| US 2002197676 | A1 | CIP of | US 1999-418529 19991014 |
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CIP of
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| PATENT NO | KIND | PATENT NO |
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| EP 1385967 | A2 Based on | WO 2002068459 |
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| US 2006035330 | A1 Cont of | US 6969597 |
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| PRIORITY APPLN. INFO: | US 2001-6922 | 20011204; US |
| | 2001-270983P | 20010221; WO |
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